and the remarks included herein show the present claims to be allowable. Therefore, applicant respectfully requests that this RESPONSE be entered and considered on its merits.

Claims 44 to 53, 58 to 63, 66 to 78, 81, 82, 84, 85, 87, 88, 95, 96, 98, 99, 101 and 102 have been canceled, without prejudice.

Claims 42, 43, 54 to 57, 79, 80, 89 to 91, 100, 103 to 105, 107 to 109 and 110 to 112 have been rejected under the judicially created doctrine of obviousness-type double patenting over claims 1 to 10 of U.S. Patent 5,919,665.

Enclosed is a Terminal Disclaimer, and required fee, disclaiming the portion of the term of any patent issuing from the above-identified application which extends beyond the full statutory term of U.S. Patent 5,919,665.

In view of this terminal disclaimer, and required fee, applicant requests that the obviousness-type double patenting rejection of claims 42, 43, 54 to 57, 79, 80, 89 to 91, 100, 103 to 105, 107 to 109 and 110 to 112 over claims 1 to 10 of U.S. Patent 5,919,665 be withdrawn.

Rejection of Claims 42, 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94, 100, 103 to 105 and 107 to 109 Under 35 U.S.C. §103(a)

The Examiner maintains the rejection of claims 42, 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94, 100, 103 to 105 and 107 to 109 based on 35 U.S.C. §103(a) as being unpatentable over Thompson et al (Eur. J. Biochem. 1990, Vol. 189, pp. 73-81), Dobeli et al (U.S. Patent No. 5,310,663) and Ford et al (Protein Expression and Purification 1991, Vol 2, pp. 95-107). The Examiner states that motivation to combine the prior art references exists because Thompson et al teaches the entire amino acid sequence of the C botulinum type A neurotoxin (BoNT/A) as deduced by nucleotide sequences analysis of the encoding gene. The Examiner makes specific reference to the last paragraph of Thompson et al where it is stated that the availability of the BoNT/A gene sequence will

allow for the production of toxin for clinical use and toxoid for the formulation of improved vaccines. The Examiner also states that there is a strong motivation for the person with ordinary skill in the art to generate a recombinant fusion protein comprising a portion of C botulinum type A toxin in view of the well-known method of constructing a fusion protein as taught by Ford et al and Doideli et al.

Applicant traverses this rejection and disagrees with the Examiner.

In the rejection the Examiner appears to be indicating that applicant's argument of non-obviousness is made primarily against whether it is obvious to make soluble recombinant botulinum toxin proteins. In fact, applicant's arguments have primarily been directed to the non-obviousness of how to make the soluble recombinant toxins. Dobeli et al and Ford et al relate to specific affinity purification tags which are used for purifying recombinant proteins. Dobeli et al and Ford et al do not disclose, teach or even suggest the production of a soluble protein when only an insoluble protein has previously been produced. Thompson relates to the nucleotide sequence and the deduced amino acid sequence of botulinum toxin type A. Combining these references does not make obvious the production of a soluble recombinant botulinum toxin protein as is described in the present patent application. The expression systems (discussed in part below in response to the 35 U.S.C §112, first paragraph rejection) employed in the present invention are not disclosed in the cited prior art and therefore no motivation can exist to combine the references to produce the present invention.

It is an important point that even if motivation to combine these references does exist, combining the references will not provide a reasonable expectation of success for the claimed method. For example, the present claims are drawn to soluble, recombinant *Clostridium botulinum* toxin proteins. The Ford et al. and Dobeli et al references offer no guidance for overcoming the problem of insolubility in recombinant proteins. As has been explained in the

previous Office Action response, the discovery of the present invention of recombinant *Clostridium botulinum* toxin proteins in soluble form (greater than 90%) is in contrast to what had been known in the art at the time of the invention (See, for example, LaPenotiere *et al.* (Toxicon 1995, Vol 33: 1383-1386). Furthermore, the references when combined do not teach each element of the claims. For example, overcoming the problem of insolubility when producing a recombinant protein of any type (not just botulinum toxin proteins) is not disclosed in any of the cited references.

The Examiner also states that "the claimed invention is a product, which has the same functional structural and function properties as [it] is disclosed in the prior art" and that the method of making the product disclosed in the present application "does not make the claimed product structurally and functionally distinct from the product disclosed in the cited prior art."

The invention as claimed does not have the same structural and/or functional properties as disclosed in the cited prior art. For example, as discussed extensively in the previous Office Action response, LaPenotiere et al (Toxicon 1995, Vol 33: 1383-1386) report the production of an insoluble recombinant (MPB)-botulinum C-fragment fusion protein. This is in contrast to the soluble recombinant protein of the present invention. The added functional feature of solubility is not disclosed in LaPenotiere et al. Further, it is well known in the art that a soluble protein is structurally different from a protein that has the same primary structure (amino acid sequence) but is insoluble or produced as inclusion bodies. For example, secondary and tertiary structures which result from protein folding are known to be important in determining solubility of recombinant proteins.

Furthermore, the Examiner states on page 5, last paragraph of the Office Action that "it is always unpredictable the solubility of a fusion protein when it is finally expressed." The Examiner's admission of unpredictability supports applicant's contention of

non-obviousness. MPEP 2143.02 states that "at least some degree of predictability is required" for a finding of obviousness. In particular, with no predictability, there can be no reasonable expectation of success, which is a requirement for a finding of obviousness.

Applicant has demonstrated that the rejected claims are non-obvious and requests that the present rejection be withdrawn.

Rejection of Claims 42 and 43 Under 35 U.S.C. §102(b)

The Examiner newly rejects claims 42 and 43 under 35 U.S.C. §102(b) as anticipated by Moberg et al and LaPenotiere et al. The Examiner states that Moberg et al, which discloses a method for using affinity chromatography to purify a botulinum type A toxin, and LaPenotiere et al, which discloses a method to recombinantly express and isolate a fragment of botulinum toxin type A, each anticipate the rejected claims. Applicant traverses this rejection.

Moberg et al discloses the purification of a native botulinum toxin type A. The reference does not disclose, teach or even suggest a soluble recombinant botulinum toxin. For example, the present specification defines "soluble", when used in reference to a recombinant protein, as "a protein which exists in solution in the cytoplasm of the host cell" (specification, page 19, lines 7 to 9) which corresponds to the accepted meaning of the term in the art. In addition, the protein of the present invention is a recombinant protein which is not the same as a native protein which may be disclosed in Moberg et al. Moberg et al does not disclose, teach or even suggest a recombinant botulinum toxin protein which exists in solution in the cytoplasm of a host cell. Therefore, a soluble recombinant botulinum toxin has not been disclosed in Moberg et al, and claims 42 and 43 are not anticipated by Moberg et al under 35 U.S.C. 102(b).

LaPenotiere et al discloses recombinant production of an

botulinum type A C-fragment fusion protein produced in insoluble form (inclusion bodies). LaPenotiere et al does not disclose, teach or even suggest a soluble recombinant botulinum toxin. It is well known in the field of molecular biology and biochemistry that a soluble protein is structurally different from an insoluble protein. For example, it is known that a soluble protein may not be folded (involving secondary and/or tertiary structures) the same as an insoluble protein. Therefore, LaPenotiere et al does not anticipate claims 42 and 43 under 35 U.S.C. 102(b).

Rejection of Claims 54, 93 and 110 to 112 Under 35 U.S.C. §112, first paragraph (Written Description)

The Examiner rejects claims 54, 93 and 110 to 112 under 35 U.S.C. §112, first paragraph. The Examiner makes a written description rejection stating that only certain amino acid sequences are disclosed in the specification and that the metes and bounds of the sequences encompassed by the invention are not set forth. The Examiner cites University of California v. Eli Lilly and Co. (Fed. Cir. 1997). Applicant traverses this rejection.

Applicant submits that the present claims are directed to sequences that are well known in the art at the time of filing. As is stated in the present specification on page 39, line 28 to 29: "*C. botulinum* toxin genes from all seven serotypes have been cloned and sequenced (Minton (1995), *supra*)". Thus, the amino acid sequence information is inherent in the specification. A practitioner of ordinary skill is able to obtain the information necessary to produce the claimed invention in accordance with the enabling disclosure provided in the specification. Furthermore, as is discussed in detail on pages 9 and 10 of the present Office Action response, specific guidance is given in the specification as to portions of a botulinum toxin that can be used for a vaccine.

The University of California v. Eli Lilly and Co., cited by the Examiner is not applicable in the present case. In Eli Lilly, the court ruled that a claim cannot be made to an unknown cDNA sequence by an applicant who has only disclosed a potential method of isolating such a sequence, even though the method has been used by the applicant to isolate a homologous cDNA sequence from a different organism. The facts in Eli Lilly are in contrast to those in the present patent application where protein sequences in question were well known in the art prior to the filing date of the above-identified application.

In view of the above, applicant respectfully requests that the written description rejection of claims 54, 93 and 110 to 112 under 35 U.S.C. 112, first paragraph be withdrawn.

Rejection of Claims 42 to 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94 100, 103 to 105, 107 to 112 Under 35 U.S.C. §112, first paragraph (Enablement)

The Examiner rejects claims 42, 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94 100, 103 to 105, 107 to 112 under 35 U.S.C. §112, first paragraph. The Examiner rejects the claims based on enablement stating that the specification only teaches certain fragments of type A toxin are suitable to be expressed as a soluble fusion protein. Applicant disagrees with the Examiner and traverses this rejection.

The Examiner specifically relies on the data in Table 16 stating that the variable results obtained for solubility using the same vector demonstrate the unpredictability of a fusion protein when it is expressed. For example, the Examiner states that clones pMA660-110, pPA1100-1450 and pPA1100-1870, which produce soluble proteins, have certain *Clostridium botulinum* toxin fragments.

In response, applicant submits that pMA660-110, pPA1100-1450

and pPA1100-1870 and the other clones listed in table 16 relate to data for *Clostridium difficile* type A toxin protein expression and not to botulinum toxin protein expression. Therefore, Table 16 does not disclose results for expression of botulinum toxin proteins.

The present specification fully discloses the use of a weak promoter, among other things, including chaperone mediated folding of recombinant proteins, to produce soluble recombinant botulinum toxin protein. For example, as stated on page 132, lines 15 to 18 and page 189, lines 7 to 12, a T7 promoter sequence is used in the pETHisa vector upon which the pHisBot vector is based. As is well known in the art the T7 promoter is unlike other promoters commonly used for *in vivo* expression, such as *lac, tac* and p_1, in that the T7 promoter is not recognized by *E. coli* RNA polymerase. Therefore, when expressing a gene in *E.* coli using a T7 promoter, there is little transcription in the absence of a source of T7 RNA polymerase. A T7 RNA polymerase gene is encoded in the genome of pETHisa expression *E. coli* host cells, BL21(DE3),and is under the control of an inducible promoter providing for inducible control of the T7 promoter. Further, the present invention utilizes a pLysS strain of host cell designated BL21(DE3)pLysS (page 193, lines 7 to 9) which includes a T7 lysozyme encoding plasmid. T7 lysozyme is an inhibitor of T7 RNA polymerase. In BL21(DE3)pLysS host cells, the basal level of T7 transcription that occurs naturally in the absence of an inducer is virtually eliminated by the T7 lysozyme that is produced. In addition, production of T7 lysozyme in the host cell weakens the relative strength of the T7 promoter when the T7 RNA polymerase gene is in the induced state by reducing the level of active T7 polymerase.

This T7 lysozyme expression regulation of the pHisBot fusion protein may be a contributing factor to the production of a soluble pHisBot fusion protein. This is supported by the present specification on page 194, line 20 to page 195, line 4, where it is

stated that a large scale purification of pHisBot was performed using a BL21(DE3) rather than the BL21(DE3)pLysS strain of *E. coli*. As expected, the deletion of the pLysS plasmid in the BL21(DE3) host yielded higher levels of expression than in the pLysS containing host strain. However, yield of soluble fusion protein was very low in the BL21(DE3) strain which yielded the fusion protein as insoluble inclusion bodies. This is in contrast to the production of a fusion protein with greater than 90% solubility when the pHisBot construct is expressed in the BL21(DE3)pLysS host strain.

In addition, chaperone mediated folding can be utilized to assist in producing soluble recombinant botulinum proteins as described in, for example, Example 32 beginning on page 211 of the specification and in Dr. Williams' declaration.

The present specification describes not only the production of a soluble type A C-fragment, but also, for example, the production of soluble recombinant type B (Example 35), C (Example 46), D (Example 47), E (Example 41), F (Example 48) and G (Example 49) C-fragments which demonstrates the usefulness of the described systems for producing soluble botulinum toxin proteins in addition to the C-fragment of botulinum toxin type A.

The Examiner states in the last paragraph of page 3 in the present Office Action that the system described in the present specification "may be a method for making the product". Applicant agrees that a method for making the claimed product is included in the specification; and therefore, applicant submits that the production of the claimed product is enabled.

The Examiner also states in this rejection that the specification is deficient for teaching how to select fragments to express and that therefore the scope of the claims is too broad.

In response, applicant submits that guidance is presented in the present specification as to the portions of a botulinum toxin that can be used for a vaccine. On page 19, lines 13 to 23, the

specification states that vaccines refer to compositions "which are capable of provoking an immune response in a host animal" directed against one (monovalent vaccine) or more (multivalent vaccine) types of clostridial toxin. The specification further discloses in examples that provoking an immune response means inducing the production of antibodies which protect the host against challenge with a toxin.

Specific guidance is given in the present specification for determining which portions of a *Clostridium botulinum* toxin provoke an immune response. For example, the specification teaches that an immune response to *Clostridium botulinum* type A toxin may be characterized using a mouse neutralization assay. In addition, the specification states that "[t]he mouse model is the art accepted method for . . . evaluation of antibotulinal antibodies." (page 165, lines 30 to 31). Further, as discussed in some detail in item 7 on page two of Dr. Williams' declaration which was filed with the previous Office Action response, the mouse neutralization assay was used to determine the biological activity of certain botulinum toxin fragments disclosed in the specification. Therefore, the mouse neutralization assay is recognized as a suitable method for determining the function in terms of biological activity of any immunogen (including any "portion" of a *Clostridium botulinum* toxin) as a vaccine.

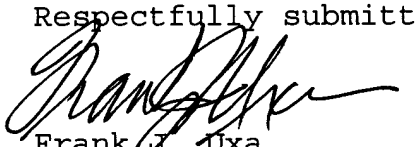
The present specification makes clear that the present invention contemplates the production of soluble botulinum toxins, botulinum toxin fusions and fusion proteins comprising portions of botulinum toxins. For example, it is stated on page 45, lines 23 to 24, that "The subject invention provides methods which allow the production of soluble *C.* botulinum toxin proteins in economical host cells;" on page 41, lines 11 to 12, that "the invention contemplates the expression of any of the botulinal toxins (*e.g.*, types A-G) as soluble recombinant fusion proteins" and on page 40, lines 15 to 18, that "The present invention contemplates fusion

proteins comprising portions of *C. botulinum* toxins from serotypes A-G." It is within the skill of an ordinary technician in the field to produce these proteins by employing the expression system described in the present invention.

In view of the above, applicant requests that the enablement rejection of claims 42, 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94, 100, 103 to 105, and 107 to 112 under 35 U.S.C. 112, first paragraph be withdrawn.

In conclusion, applicant has shown that the present claims are not anticipated by and are unobvious from and patentable over the prior art under 35 U.S.C. 102 and 103. In addition, applicant has shown that the written description and enablement requirements under 35 U.S.C. 112 have been satisfied. Therefore, applicant submits that claims 42, 43, 54 to 57, 79, 80, 83, 86, 89 to 91, 93, 94, 100, 103 to 105 and 107 to 112 are allowable and respectfully requests the Examiner to pass the above-identified application to issuance at an early date.

Respectfully submitted,



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